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Plasmid constructs and plant transformation

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An abbreviated version of this protocol was published in Science Advances in Mar 2022

An MKP-MAPK protein phosphorylation cascade controls vascular immunity in plants

DOI: 10.1126/sciadv.abg8723

Detailed protocol

To make CRISPR-Cas9 knockout constructs, 20-bp gene-specific guide RNA sequences targeting MKP1and MYB4 in Arabidopsis were fused to pCAMBIA1300-pYAO:Cas9 and sgRNAs targeting exons of OsMKP1, OsMPK3, OsMPK6, OsMYB102 and OsMYB108 in rice were fused to pYLCRISPR/Cas9-MT. For overexpression constructs, the coding sequence (CDS) sequences were inserted into pCambia1300-*MYC* or pCambia1300-*GFP* (green fluorescent protein) vectors for Arabidopsis transformation or cloned into PUN1301-*pUBI-Flag* vector driven by the maize UBIQUTIN1 promoter for rice transformation. The constructs were introduced into *Agrobacterium tumefaciens* strain GV3101 (for Arabidopsis) and EHA105 (for rice) and then transformed into different genetic backgrounds to produce more than 15 independent transgenic lines for each construct. Further selection and validation were based on PCR-based sequencing or Western blotting.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. He, Z. (2022). Plasmid constructs and plant transformation. Bio-protocol Preprint. bio-protocol.org/prep2050.
2. Lin, H., Wang, M., Chen, Y., Nomura, K., Hui, S., Gui, J., Zhang, X., Wu, Y., Liu, J., Li, Q., Deng, Y., Li, L., Yuan, M., Wang, S., He, S. Y. and He, Z.(2022). An MKP-MAPK protein phosphorylation cascade controls vascular immunity in plants. Science Advances 8(10). DOI: 10.1126/sciadv.abg8723